



## The effect of nitrate addition on abundance of *nirK*, *nirS* and *gln* genes in acidified Norway spruce forest soil

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The denitrification is the main biotic process leading to losses of fixed nitrogen as well as removal of excess of nitrate ( $\text{NO}_3^-$ ) from the soil environment. The reduction of  $\text{NO}_2^-$  to nitric oxide (NO) distinguishes the “true” denitrifiers from other nitrate-respiring bacteria. This reaction is catalyzed by two different types of nitrite reductases, either a cytochrome cd1 encoded by *nirS* gene (*nirS* denitrifiers) or a Cu-containing enzyme encoded by *nirK* gene (*nirK* denitrifiers). The *nirS* denitrifiers are located mostly in rhizosphere, while the *nirK* denitrifiers are more abundant in bulk soil. These two groups can be also classified as markers of denitrification.

Glutamine synthetase is one of the main bacterial  $\text{NH}_4^+$  assimilating enzymes; it is coded by *glnI* gene. Glutamine synthetase is mostly active when N is the limiting factor for bacterial growth. There is recent evidence that the activity may be affected by the presence of alternative N source (i.e.  $\text{NO}_3^-$ ). However, in anaerobic condition  $\text{NO}_3^-$  can be used also by the denitrifying bacteria so there may be strong competition for this nutrient.

The laboratory experiment was performed to evaluate the effect of nitrates ( $\text{NO}_3^-$ ) on abundance of *nirK*, *nirS* and *gln* gene copy numbers. The amount of  $\text{NO}_3^-$  corresponded to the actual atmospheric depositions on experimental sites in the Bohemian Forest. Litter organic layer (0-5cm of soil) was used for laboratory incubation experiment. Four replicates of control (no addition of  $\text{NO}_3^-$ ), and  $\text{NO}_3^-$  addition were incubated anaerobically for one month. After the incubation DNA was extracted and the number of *nirK*, *nirS* and *gln* gene copies was determined using qPCR (SYBRGreen methodology). Results showed that the addition of  $\text{NO}_3^-$  significantly increased the number of *nirK* and *nirS* denitrifiers from  $5.9 \times 10^6$  to  $1.1 \times 10^7$  and from not detectable amount to  $1.4 \times 10^6$ , respectively. The *gln* gene copy number was also higher after  $\text{NO}_3^-$  addition. However, the difference was not statistically significant.